

Fig. 4A

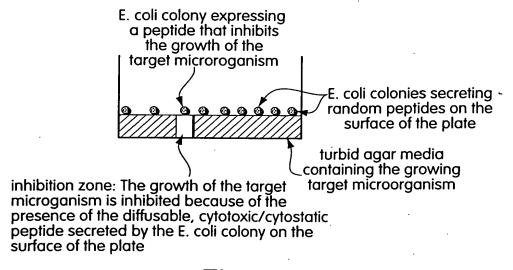


Fig. 4B



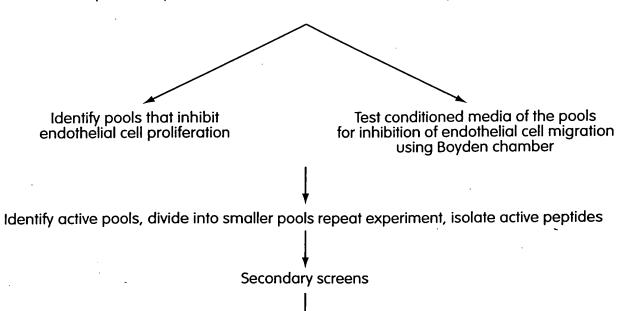
Construct a peptide library using the M13/COS phagemid vectors

Use the library in M13 peptide "display mode to isolate peptides that bind to capillary endothelial cells

Incubate the peptides that bind to endothelial cells with fibroblasts. Collect peptides that can not bind to fibroblast cells

Titrate, pool and ampilify the isolated peptides

Express the pools in COS cells ("secretion mode") using Transwells



Test anti-angiogenic activity of the synthetic peptides in vivo

Repeat primary and secondary screens using synthethic peptides

Fig. 5

JUL 2		GAATTGTGAGGGGATAACAATTTCTA L L E. coli mRNA Start	lg branch and splice acceptor	ггтзтгрвяр	e) (ijo	BstXI (1) Notl Myc epitope	rcggargggcggcggcaaaagcrc G W A A A E Q K L 1 G W A A A E Q K L		MIJSpVIII	CGATCCCTTCACCTCGAAAGCAAGCTGA D P F T S K A S E. coli Stop	SV40 polyA	
pAM6	lac promoter	CGCAATTACTGTGAGTTAGCTCATTAGGCACCCCAGGCTTTACACTTTATACTTCCGGCTCGTATATTGTGTGGAATTGTGAGCGGATAACAATTTCTA -35 box -10 box E. coli mRNA Start	lg branch and splice acceptor GAAGGAAACHAGTAAGTAATTATTATTATTATTCGCAATTCCTTTAGTTGTTCTTATTCTCACTCGCTGAATTACTGCCTTTGCCTTTCCCA	A I P L V V P F Y S H S A E	signal peptidase cleavage site (E.coli)	IgH secretion signal (mouse)	CAGGGGGCCACCACGATGGGGTTATCTTCTTGCTGATGGCAGTGGTTACAGGGGTCAATTCAGCACCAGAGGGGGGGG	signal peptidase cleavage site (eukaryotic)	globin splice Pstl BstXI (2) donor	ATTICTGAAGAGGACTIGGCACCATCACCATCACCATTGCCAGGTAAGTGCTGAGGGTGACGATCCCTTCACCTCGAAAGCAAGCTGAAGCTGAAGCAAGCTGAAGCAAGC	E. coli transciptional terminator Ig branch and splice acceptor TAAAGTCTAAAGCCCGCCTAATGAGCGGGCTTTTTTTACTGACA test collega gaggccTTTCTCTCCACAGGGGTAGATAACTGAACTTATTACTAGATTATAATG	
·	end of CMV	CGCAATTACTGTGAGTTAGCTCACTCATTAGGCACC	lac promoter GAAGGAAACAGCTAAGTAtgAAAAAATTATTGG	RBS globin splice ► K K L L F donor	E. coli Met	Kozak	CAGGGGCCACCAtgAAATGCAGCTGGGTTATCTTC Q G A T A K C S W V I F	eukaryotic Met	Myc epitope 6xHis tag	ATTICTGAAGAGACTTGGCACACCATCACCATCACIS STATES	E coli transciptional terminator TAAAGTCTAAAGCCCGCCTAAATGAGCGGGCTTTTTTT	

Fig. 6

JUL 2 6 2002 & JUL 2 6 ZOOZ AS

GGCACCCCAGGCTTTACACTTTATACTTCCGGCTCGTATATTGTGTGGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGAAAATCAAACTGGCG E. coli Stop SV40 polyA --TTCACCTCGAAAGCAAGCTGA lac promoter PTACTCGCCCTGACTTCTCTTTCTGCTCTTGCAGGTCCAGGCGGCGGCGCCGCAGGAAAGCTCATTTCTGAAGAGGACTTGGCACATCACATCAC L L A L T S L S A L A G P G G W A A A E Q K L I S E D L A H H H ល E SO prokaryotic Starl globin splice do nor signal peptidase cleavage Myc epitope site (eukaryotic) M13 pVIII z > ڻ lg branch and splice acceptor lac promoter E. coli mRNA Start ğ CATCACCATCTGCAGCCATTATCTTGGCAGGTAAGTGCTGAGGGTGACGATCCC---IgH signal peptide Ω Ω Ö BstXI(1) signal peptidase cleavage ы S BsfXI (2) globin splice donor site (E. coli) > E. coli transciptional terminator Ig branch and splice acceptor O U eukaryotic Start × 3 "designer" secretion signal end of CMV Xbal Kozak lac operator

eukaryotic Stops

X Fool

TAAAGTCTAAGCCCGCCTAATGAGCGGGCTTTTTTTTTACTGACatcctcgaggCCTTTCTCTCTCCACAGGGGTAGATAACTGAACTTGTTTATTGCAGATTATAATG...

E. coli/eukaryotic signal peptidase cleavage site

E. coli/eukaryotic

Met

PARTE	JUL 2 6 2002	C48 355
	pa]	— 1

pAM8

GAAGGAAAGCCACCatgTCTATCCAACACTTCCGTGTTGCATTAATCCCTTTCTTTGCAGCGTTCTGTTTACCTGTTTTCGCAGGTCCAGGCGGATGGGCGGCGGC ¥ V BstX (1) G lac operator Ö E. coli mRNA Start > -10 box ы U Ŀ 4 **B-lactamase signal sequence** lac promoter щ -35 box ĸ Kozak end of CMV

GAGCAAAAGCTCATTTCTGAAGAGGACTTGGCACACCATCACCATCACCATCTGCAGCCATTATCTTGGCAGGTAAGTGCTGAGGGTGACGATCCC---TTCACC M13 pVIII 回 globin splice Ø donor O ≊ BstXI (2) Ø Pstl н I H 6xHis tag H Ħ Ħ 闰 Myc epitope M O

eukeryotic Stops lg branch and splice acceptor Xhol E. coli transciptional terminator E. coli Stop 4 ×

SV40 polyA



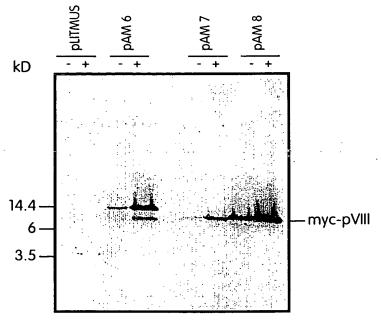
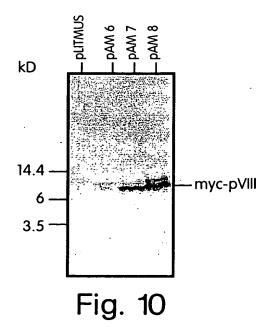


Fig. 9





Characterization of the peptide display vectors

<u>Plasmids:</u>	<u>c.f.u.</u> :	<u>p.f.u.</u> :	<u>c.f.u./p.f.u</u> .:
pAM6	2.8x10 ¹² /ml	1.9x10 ¹¹ /ml	~15
pAM7	2x10 ¹² /ml	1.5x10 ¹¹ /ml	~13
8MAq	10 ¹² /ml	2.2x10 ¹¹ /ml	~5
pLITMUS	2x10 ¹² /ml	8.4x10 ¹¹ /ml	~2

Fig.11

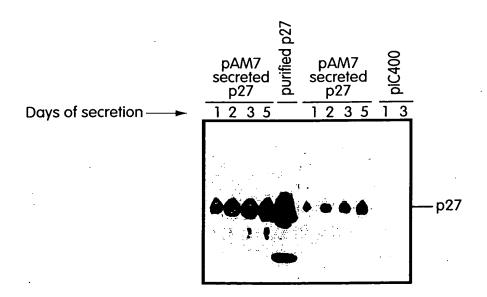


Fig. 12



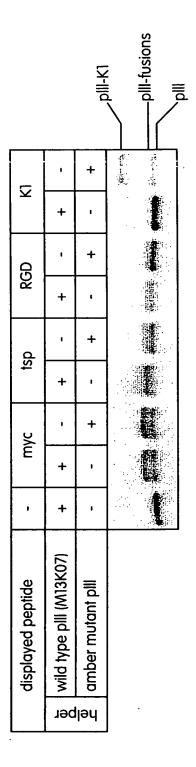


Fig. 13



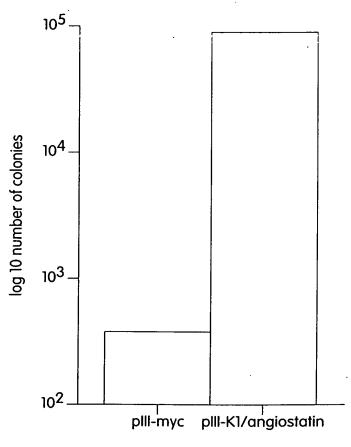


Fig. 14



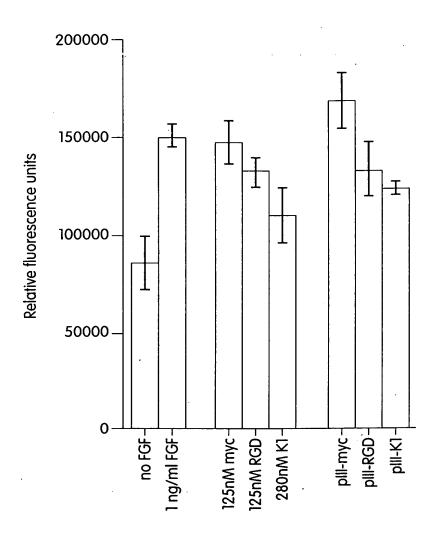


Fig. 15A



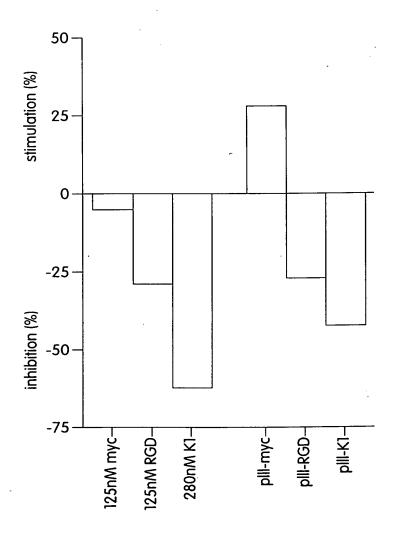


Fig. 15B